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Evaluation of a Quantitative Photometric Latex Agglutination Immunoassay for α-Foetoprotein

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Summary: A simple quantitative photometric method is described for the determination of serum α-foetoprotein using latex particle agglutination in an immunochemical system. This method is based on the quantitative photometric measurement of agglutination of latex particles coated with antibodies against α-foetoprotein. The turbidity is measured at a wavelength of 340 nm. Agglutination causes a decrease in absorbance. Interference by serum constituents, e. g. rheumatoid factors, are avoided by pretreating the serum samples with buffered polyethylene glycol. Concentrations of 0 to 640 μg/l were used for the standard curve. Analytical recoveries were 99.5 to 105.2%. Maximum within and between runs coefficients of variation were 6.2 and 11.6%. The correlation coefficient of the method with radioimmunoassay (RIA), calculated from results on 117 serum samples, was 0.997, and the regression equation y = 0.99x (RIA) − 7.23.

Introduction

α-Foetoprotein (AFP) is a glycoprotein of molecular weight 70 000. During embryogenesis it is first synthesized by the yolk sac and later by the foetal liver of mammals (1–3). Trace synthesis of α-foetoprotein still continues in children and adults, and low levels of α-foetoprotein are detectable in their sera (4).

During pregnancy, the quantitation of embryonal α-foetoprotein in maternal serum and amniotic fluid has been shown to be useful in the detection of various foetal disorders, including neural tube defects (5–8) and congenital nephrosis (9–11). In such disorders α-foetoprotein concentrations are generally increased. In the non-pregnant state raised serum α-foetoprotein levels are associated with various liver diseases e. g. primary and secondary liver cancer (12, 13), and tumours, including yolk sac structures (12). In these cases α-foetoprotein determination may serve as a diagnostic aid and/or therapeutic monitor.

In the numerous commercially available test kits for α-foetoprotein radioimmunoassay (RIA) is the most widely used method. RIA, however, requires a special laboratory with complicated and expensive instrumentation. In this paper a photometric latex agglutination immunoassay for α-foetoprotein is evaluated using commercially available reagents. This method enables the quantification of serum α-foetoprotein with a normal photometer, an instrument found in most laboratories.

Materials and Methods

Serum samples

For α-foetoprotein (AFP) determination, 97 serum samples from normal and pathological pregnancies and 20 samples from patients with liver diseases, which had first been analysed by radioimmunoassay (RIA-gnost^{R} AFP, Behringwerke AG, Marburg-Lahn, F. R. G.), were obtained from the Kuopio University Central Hospital, Kuopio, Finland. In addition 24 sera positive for rheumatoid factor by RapiTex^{R}-RF (Behringwerke AG) and by Waaler-Rose, obtained from United Laboratories, Helsinki, Finland, were analysed in order to study nonspecific agglutination and its elimination.

Reagents

Lyophilized AFP latex reagent in 0.1 mol/l glycine-buffered saline, containing bovine serum albumin 20 g/l, pH 9.2 (anti-AFP coated latex particles) (14).

Lyophilized AFP-references: delipidized zero level bovine serum and high level serum, 640 µg/l standardized against WHO AFP preparation 72/225.

Sample pretreatment solution containing polyethylene glycol (PEG 6000) 84 g/l in 0.01 mol/l phosphate-buffered saline, pH 7.4.
Diluting buffer containing Tween 20 0.5 g/l in 0.1 mol/l glycine-buffered saline. All these reagents were supplied by Orion Diagnostica, Espoo, Finland.

Preparation of α-foetoprotein references

Lyophilized zero level and high level reference were reconstituted with 2 and 1 ml of distilled water, respectively. For the standard curve the high level reference was diluted with zero level reference to obtain the concentrations of 0, 10, 20, 40, 80, 320 and 640 µg/l.

Specimen preparation

Serum samples and references were diluted 5-fold with pretreatment solution, mixed well, allowed to stand and centrifuged for 5 min (1500 *g*). The supernatant was used in the assay.

Assay procedure

Pretreated reference or sample (25 µl) was pipetted into the test tubes. Latex reagent (50 µl) was added, followed by gentle mixing by hand. After incubation for 30 min at room temperature, 2 ml of diluting buffer was gently added to the tubes. After at least 45 min, the absorbances were measured at a wavelenght of 340 nm against water.

Calculation of α-foetoprotein concentration

The absorbance differences (Δ A) between zero level reference and other tubes were calculated. A graph with Δ A for the references on the Y-axis and the concentration of the references on the X-axis (logarithmic scale) was plotted and the sample concentrations taken from this curve. If the α-foetoprotein value was higher than that of the highest standard, the sample was diluted with a zero level reference and reanalysed.

Instrumentation

Gilford Stasar III spectrophotometer with a low volume flowthrough cuvette, Gilford Instrument Laboratories Inc. Oberlin, OH 44074, USA.

Results

Reference curve

A typical nonlinear reference curve in the α-foetoprotein range of 10—640 µg/l is shown in figure 1. The antigen excess phenomenon was seen at α-foetoprotein concentration of 5000 µg/l, and thereafter the curve declined slowly so that Δ A of the highest standard was not reached at 40 000 µg/l (tab. 1). The time needed for end-point agglutination was 15—20 min but the reaction time could be extended to 60 min without any influence on the results.

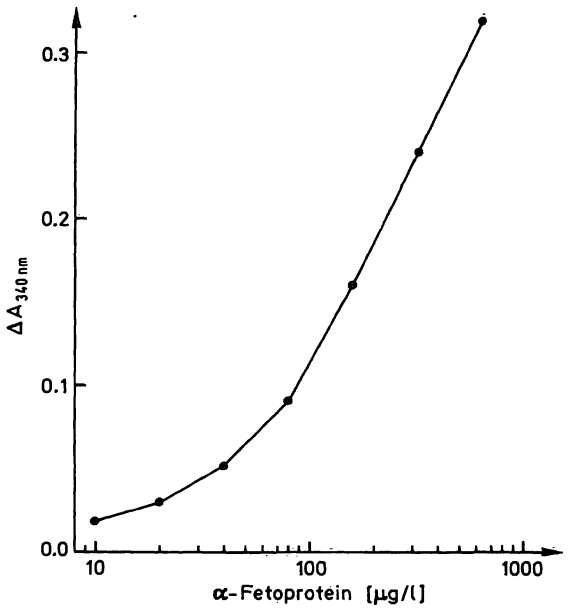

Fig. 1. Reference curve for the determination of α-foetoprotein by quantitative photometric latex agglutination immunoassay.

Tab. 1. Linearity of photometric latex agglutination immunoassay for α-foetoprotein.

Dilution	α-Foetoprotein (µg/l)		
	in dilution	final	mean
1:1	> 640		
1:2	> 640		
1:4	> 640		
1:8	> 640		
1:16	> 640		
1:32	> 640		
1:64	550	35 200	
1:128	280	35 800	
1:256	130	33 300	
1:512	78	39 900	
1:1024	43	44 000	
1:2048	20	41 000	38 200

Linearity

The linearity of the method was tested by serially diluting a patient sample with the zero level reference. Different dilutions gave the final results ranging from 33 300 to 44 000 µg/l with the mean α-foetoprotein level of 38 200 µg/l (tab. 1).

Analytical recovery

α-Foetoprotein recovery after addition to three serum samples, with theoretical concentrations covering most of the range of the standard curve, varied from 99 to 105% (tab. 2).

Reproducibility

The within run precision (CV) was 6.2% at low and 5.1% at high α-foetoprotein concentrations, whereas the between run precision was 11.6% at low and 6.5% at high α-foetoprotein concentrations (tab. 3).

Elimination of serum interference

Nonspecific macromolecular agglutinators in serum were eliminated by sample pretreatment with polyethylene glycol containing buffer. Table 4 shows agglutination data caused by rheumatoid factors, with predilution of sera by pretreatment solution and saline. False positive results were seen when those sera were diluted with saline. By using polyethylene glycol, nonspecific agglutinations were effectively eliminated.

Comparison with RIA

A total of 117 serum samples, previously analysed by RIA and stored at −20 °C, were determined by this method. The coefficient of correlation was 0.997 and the regression equation y = 0.99x (RIA) − 7.23 (fig. 2).

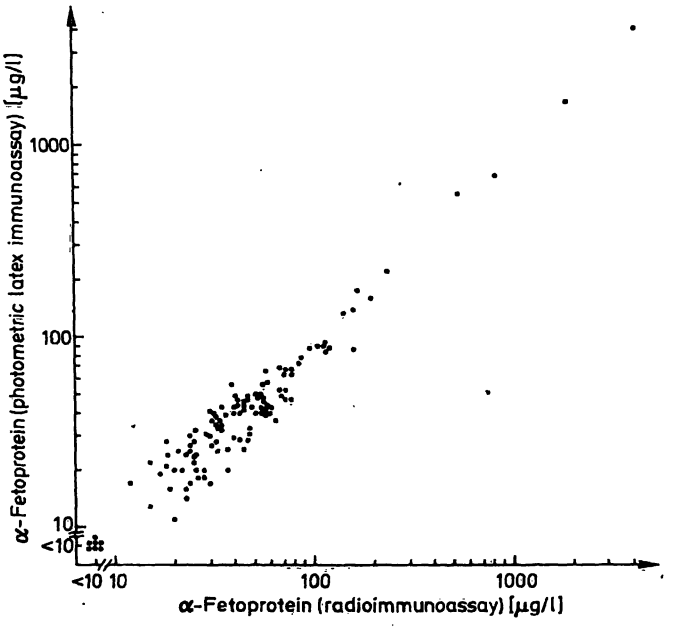

Fig. 2. Correlation between radioimmunosassay (RIA) and photometric latex agglutination immunoassay for α-foetoprotein.
r = 0.997, y = 0.99x (RIA) − 7.23, N = 117.

Tab. 2. Analytical recovery of α-foetoprotein in photometric latex agglutination immunoassay.

α-Foetoprotein (µg/l)					
Added	Recovered		Recovery (%)		n
	Mean	SD	Mean	SD	
344	349.1	10.48	101.5	3.1	10
114	113.4	4.76	99.5	4.1	10
23	24.2	1.31	105.2	5.7	10

Tab. 3. Reproducibility of photometric latex agglutination immunoassay for α-foetoprotein.

	α-Foetoprotein (µg/l)		CV, %	n
	Mean	SD		
Within run*)	43	2.7	6.2	30
	248	12.6	5.1	30
Between run**)	34	3.9	11.6	10
	263	17.0	6.5	10

*) Determinations within a day with the same latex suspension.
**) Determination on different days with different latex suspensions taken from the same lyophilized batch.

Tab. 4. Influence of rheumatoid factors on agglutination and elimination of this interference by sample pretreatment.

Sample*) diluted 5-fold in	Response, decrease in absorbance		Corresponding α-foetoprotein (µg/l)	
	Range	Mean	Range	Mean
Saline	0−0.315**)	0.117**)	0−650***)	82***)
Pretreatment solution	0−0.021	0.006	0− 14	2

*) Twenty four sera, all positive in RF-latex test and *Waaler-Rose* titres ranging from 45 to 5600, median 240, were analysed.
**) Nonspecific agglutination.
***) False positive results.

Discussion

The first photometric latex agglutination immunoassay for rheumatoid factors has already been described (15). More recently this method has been developed for determination of β_2-microglobulin in human urine and serum (16), of C-reactive protein (17) and of ferritin (18) in human serum. Now it has been applied to the determination of α-foetoprotein in human serum. Also, another latex agglutination method, called a particle counting immunoassay, has been adapted for the determination of α-foetoprotein (19).

Many interferences by serum, especially rheumatoid factor, can interfere in any immunoassay. To avoid these interferences in latex aggluctination tests, various methods have been used (15, 19, 20, 21, 22). False positive values due to rheumatoid factor in untreated sera were also seen in this work. The non-specific agglutination was successfully eliminated by sample pretreatment with buffered polyethylene glycol.

The antigen excess area was at an α-foetoprotein level beyond 5000 µg/l. However, values up to 40 000 µg/l gave a higher decrease in absorbance than the highest standard. References of zero to 640 µg/l cover the most important measuring range for monitoring pregnancies at risk (6, 23, 24), and for diagnosing and monitoring hepatoma and teratoma (12, 13) as well as benign liver diseases, cirrhosis and hepatitis (25–28).

In primary liver cancer serum α-foetoprotein concentration may vary from the normal value (below 20 µg/l) (4) up to 840 000 µg/l (13). In cases of suspected liver cancer serial serum dilutions must be used to avoid the risk of missing such high α-foetoprotein concentrations due to the antigen excess.

The photometric latex agglutination immunoassay evaluated in this paper is simple to perform and relatively rapid, and it is suitable for automation. Manually, it is possible to analyse 50 sera in half a day. The dilution linearity was excellent. Many disadvantages of radioisotopes in RIA or enzyme-conjugates in ELISA are avoided with the use of unlabelled latex reagents, which can be lyophilized, and are stable for years. The most important advantage is the ability to measure AFP with a normal spectrophotometer, equipment available in most laboratories. Instrumentation for RIA or particle counting is more complicated and expensive. The specificity, sensitivity and precision of the results obtained by the quantitative photometric latex agglutination immunoassay for α-foetoprotein are as good as those obtained by RIA techniques.

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